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ARMSTRONG LABORATORY

STORAGE STABILITY OF pCTFE CARBOXYLIC ACID METABOLITES

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

JAMES N. McDOUGAL, Lt Col, USAF, BSC
Deputy Director, Toxicology Division
Armstrong Laboratory

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Studies conducted at the Toxic Hazard Research Unit have analyzed trimer and tetramer carboxylic acid metabolites of polychlorotrifluoroethylene (pCTFE) in biological samples. Tissue samples have been stored at -70 °C, and urine and feces have been stored at -20 °C for periods of time ranging from several months to over 1 year. At the time of these analyses, the storage stability of the carboxylic acid metabolites of pCTFE was not known. To investigate the storage stability of the carboxylic acid metabolites of pCTFE, male Fischer 344 rats were dosed orally with pCTFE parent compound and sacrificed after 48 h. Samples of urine were collected from 24 to 48 h after dosing, and liver, kidney, lung, and blood were collected at 48 h. Samples were analyzed via gas chromatography with electron capture detection. Concentrations of the carboxylic acid metabolites were measured at 2 weeks, 6 weeks, 17 weeks, and 55 weeks. The stability data were analyzed using a 1-factorial multivariate analysis of variance with trend analysis to evaluate the storage stability of the pCTFE carboxylic acid metabolites.

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PREFACE

The research reported herein was conducted by the Toxic Hazards Research Unit, ManTech Environmental Technology, Inc., and serves as a final report on the storage stability of pCTFE carboxylic acid metabolites. The research described in this report began in January 1991 and was completed in May 1992. It was performed under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. F05 [II]). Lt Col James N. McDougal served as Contract Technical Monitor for the Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory, Wright-Patterson Air Force Base, OH.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals* prepared by the Committee on Care and Use of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institutes of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

The authors gratefully acknowledge Janet L. Wilson of ManTech Environmental for her technical assistance.

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ABBREVIATIONS

C	Celsius
cm	Centimeter
CTFE	Chlorotrifluoroethylene
F-344	Fischer 344
GC	Gas chromatograph
GC/ECD	Gas chromatograph with electron capture detection
g	Gram
h	Hour
i.d.	Inside diameter
kg	Kilogram
m	meter
µg	Microgram
µL	Microliter
µm	Micrometer
mL	Milliliter
mm	Millimeter
min	Minute
M	Molar
N	Normal (concentration)
ODS	Octadecylsilane
pCTFE	Polychlorotrifluoroethylene
p	Probability
sec	Second

SECTION 1

INTRODUCTION

Carboxylic acid metabolites of polychlorotrifluoroethylene (pCTFE) were first characterized by Greene et al. (1991). The trimer carboxylic acid metabolite was isolated from urine, and the tetramer was isolated from the liver of male Fischer 344 (F-344) rats dosed with parent pCTFE. These metabolites have also been found in blood, kidney, liver, lung, and urine. Studies at the Toxic Hazards Research Unit have analyzed carboxylic acid metabolites of pCTFE in tissue samples that have been stored at -70 °C for over 1 year. Samples of urine and feces have also been stored for periods over 1 year at -20 °C. These samples have come from investigations such as oral gavage studies of pCTFE carboxylic acids in rat (Kinkead, et al., 1991a), inhalation studies of pCTFE parent compound in rat (Kinkead, et al., 1991b), and oral dosage of parent pCTFE in primates. Because these metabolism studies have required the analysis of pCTFE carboxylic acids in a variety of tissues, their storage stability has become an important question. Until now, the storage stability of carboxylic acid metabolites of pCTFE has not been investigated. The objective of this study was to examine the long-term storage stability of the carboxylic acid metabolites of pCTFE. This was done by serial analysis of individual samples by gas chromatography with electron capture detection (GC/ECD).

SECTION 2

METHODS

Test Materials

Polychlorotrifluoroethylene (CAS # 9002-83-9, MLO-87-347, Lot 87-23) was supplied by Halocarbon Products (Hackensack, NJ) through the U.S. Air Force.

Laboratory Animals

Fischer 344 rats were obtained from Charles River Labs (Kingston, NY). Six male rats, each 200 g in weight, were used for oral dosing with pCTFE. The body weights of the rats at the time of sacrifice are given in Table 1.

TABLE 1. BODY WEIGHTS OF RATS

Animal	Weight (g)
Rat 001	232.4
Rat 002	239.6
Rat 003	216.4
Rat 004	257.5
Rat 005	244.5
Rat 006	247.9

Reagents

All solvents were high performance liquid chromatography (HPLC) grade or better. Methanol and *n*-hexane were purchased from Fisher Scientific (Pittsburgh, PA). Methanolic hydrochloric acid (HCl) reagent was supplied by Supelco Inc. (Bellefonte, PA). Purified trimer and tetramer chlorotrifluoroethylene (CTFE) acids and pCTFE acid methyl esters were purchased from LSC (Technolube Products Division, Irvine, CA). These materials were used as supplied. Solid phase extraction columns with octadecylsilane (ODS) packing were obtained from B&J Baxter (McGaw Park, IL). The columns were eluted using a Vac-Elut vacuum box (Analytichem, Harbor City, CA).

Instrumentation

A Varian 3500 GC equipped with an 8100 autosampler (Varian Assoc., Sunnyvale, CA) was used for analysis in splitless injection mode. A fused silica Supelcowax-10 (30 m x 0.53 mm i.d., 1.0 µm film thickness) wide-bore capillary column (Supelco, Bellefonte, PA) was installed in conjunction with an ECD. Ultra-high-purity nitrogen was used as the carrier and the make-up gas. Carrier gas flow was set at 2.4 mL/min, and the make-up gas was set at 40 mL/min. Data were acquired with a PE/Nelson 3000

series chromatography data system (Perkin-Elmer, Norwalk, CT). Means, standard deviations, and second order polynomial curve fitting were computed using RS/1 software, Version 4.0, (BBN, Cambridge, MA) on a VAX 8530 computer (DEC, Maynard, MA). Tissue samples were homogenized using a Tekmar Tissumizer (Cincinnati, OH). A Haake-Buchler vortex mixer (Saddlebrook, NJ) was used to vortex and heat samples during derivatization.

SECTION 3

EXPERIMENTAL

Dosing and Sample Collection

Animals were dosed orally with a single 1.25-g/kg dose of pCTFE and housed in metabolism cages for urine and feces collection from 24 to 48 h post dose. Test animals were sacrificed via carbon dioxide (CO_2) asphyxiation at 48 h post dose. Liver, kidney, fat, lung, brain, testes, muscle, and blood were collected at sacrifice.

To allow for the handling of specimens, the F-344 rats were divided into two groups of three. Three rats from Group 1 had tissues analyzed at 0, 2, and 17 weeks. The three rats from Group 2 had tissues analyzed at 0, 6, and 55 weeks. Approximately 0.5 g of tissue was analyzed for pCTFE carboxylic acid metabolites on the day of sacrifice and the remaining sample was refrigerated at -70 °C. Urine and feces were refrigerated at -20 °C.

Analysis

A 0.5-g sample of feces, fat, brain, kidney, lung, muscle, testes, or liver was homogenized in 5.0 mL of methanol in a 22-mL glass scintillation vial. The samples were then centrifuged at 2000 x *g* for 20 min, and the supernatants were collected for derivatization and analysis.

Urine samples were extracted on ODS solid-phase extraction columns. The columns were conditioned with 2 mL of methanol followed by 1 mL of 0.1N HCl. Samples were acidified by adding 0.1 mL of 0.1 M HCl to 1 mL of urine. The acidified urine was applied to a column that was vacuumed to dryness. The analytes were eluted with 1.2 mL of 0.01 N sodium hydroxide (NaOH) in methanol and derivatized.

Samples were derivatized by esterification in methanolic-HCl. A 100-µL aliquot of supernatant, eluant, or whole blood was reacted with 200 µL of 3 N methanolic-HCl reagent in a 1.8-mL GC autosampler vial under a 1-mL layer of hexane. The vials were tightly capped with Teflon®-coated rubber septa and then vortexed for 16 h at 50 °C. After cooling to room temperature, the hexane layer was removed, and 1 µL of the hexane layer was injected into the GC. Samples were analyzed using a standard curve, which ranged from 0.005 to 2.5 µg/mL. The same standard curve was used throughout the study, and standard solutions were refrigerated when not in use.

Chromatography Conditions

The operating conditions for GC analysis were as follows: Initial temperature was 80 °C. The temperature rise was 3 °C/min to a final temperature of 155 °C. After holding for 10 min, the

temperature rise was 10 °C/min to a final temperature of 225 °C, held for 5 min. The detector temperature was set at 300 °C, the injector temperature at 200 °C.

Statistical Analysis

To compare the levels of pCTFE carboxylic acid metabolites from the time of the initial sacrifice up until 55 weeks, the mean of two initial concentrations (obtained 48 h after dosing) from an individual tissue and from a single animal was obtained. All serial measurements for this sample were expressed as ratios normalized (divided by) to this mean. The stability of the pCTFE carboxylic acid metabolites in a specific tissue was evaluated by comparing groups of animals using a 1-factorial multivariate analysis of variance (ANOVA) with trend analysis. Statistical significance was accepted at $p \leq 0.05$.

Results[a]

The results include data from the time of sacrifice through Week 55. The lowest concentration of the CTFE trimer and tetramer methyl ester calibration curve was 0.005 µg/mL in hexane. Because of dilutions used in the extraction procedure and sample preparation, the limit of detectability of CTFE trimer and tetramer carboxylic acid metabolites was 0.5 µg/mL in tissue extracts, and 0.1 µg/mL in blood and urine. Samples that had levels less than these were not evaluated for pCTFE carboxylic acid storage stability because these data were below the smallest concentration on the standard curve. The pCTFE carboxylic acid metabolites were below these values for fat, brain, testes, muscle, and feces. Therefore, these tissues were not included in the data for the stability study analysis. The data for the storage stability of pCTFE carboxylic acid metabolites in blood are shown in Table 2. A plot of the stability of the CTFE trimer carboxylic acid metabolite in blood is shown in Figure 1, and the CTFE tetramer carboxylic acid metabolite is shown in Figure 2.

The CTFE trimer carboxylic acid metabolite in blood showed no significant trend. The CTFE tetramer carboxylic acid metabolite in blood showed an increase up to 6 weeks. This was followed by a slight decrease at 17 weeks, and an increase at 55 weeks. The significance level of this trend was $p < 0.05$.

The results of the stability study of the pCTFE metabolites in kidney are shown in Table 3. The plots for the CTFE trimer and tetramer carboxylic acid metabolites are shown in Figure 3 and Figure 4, respectively.

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[a] The original protocol called for a 43-week sample analysis and an 80-week analysis. Instrumental problems delayed the 43-week analysis until 55 weeks, and the early cancellation of the study eliminated the 80-week assay.

TABLE 2. pCTFE CARBOXYLIC ACIDS STABILITY: BLOOD

Animal ID	Weeks Postsacrifice	Trimer Conc. in Blood (µg/mL)	Tetramer Conc. in Blood (µg/mL)	Trimer Day 0 Ratio	Tetramer Day 0 Ratio
Rat 001 Blood	0	2.590	7.475	1.061	1.038
Rat 001 Blood	0	2.294	6.921	0.940	0.961
Rat 001 Blood	2	2.351	6.795	0.963	0.944
Rat 001 Blood	2	2.214	6.654	0.906	0.924
Rat 001 Blood	17	2.747	7.622	1.125	1.059
Rat 001 Blood	17	2.898	7.527	1.187	1.046
Rat 002 Blood	0	2.406	5.934	1.018	1.020
Rat 002 Blood	0	2.322	5.698	0.982	0.980
Rat 002 Blood	2	2.144	5.502	0.907	0.946
Rat 002 Blood	2	2.365	6.131	1.000	1.054
Rat 002 Blood	17	3.031	6.763	1.282	1.163
Rat 002 Blood	17	3.278	7.390	1.387	1.271
Rat 003 Blood*	0	1.752	3.572	0.666	0.553
Rat 003 Blood	0	2.009	4.119	0.763	0.637
Rat 003 Blood	2	2.601	6.362	0.988	0.985
Rat 003 Blood	2	2.665	6.562	1.012	1.015
Rat 003 Blood	17	3.082	6.855	1.171	1.061
Rat 003 Blood	17	2.879	6.631	1.093	1.026
Rat 004 Blood	0	1.687	3.269	1.046	1.076
Rat 004 Blood	0	1.540	2.810	0.954	0.924
Rat 004 Blood	6	1.103	2.978	0.683	0.980
Rat 004 Blood	6	1.347	4.280	0.834	1.408
Rat 004 Blood	55	1.659	3.853	1.028	1.268
Rat 005 Blood	0	1.244	3.089	0.904	0.889
Rat 005 Blood	0	1.509	3.857	1.096	1.111
Rat 005 Blood	6	1.402	2.857	1.018	0.823
Rat 005 Blood	6	1.437	4.937	1.044	1.422
Rat 005 Blood	55	1.278	4.094	0.929	1.179
Rat 006 Blood	0	1.971	4.661	1.008	0.995
Rat 006 Blood	0	1.941	4.707	0.992	1.005
Rat 006 Blood	6	1.388	4.708	0.710	1.005
Rat 006 Blood	6	2.033	5.406	1.039	1.154
Rat 006 Blood	55	2.326	6.524	1.189	1.393

* Blood samples for Rat 003 were normalized using the 2-week postsacrifice samples. This was done because of a derivatization error that occurred with the 0-week postsacrifice samples. All other blood samples were normalized to the 0-week postsacrifice values.

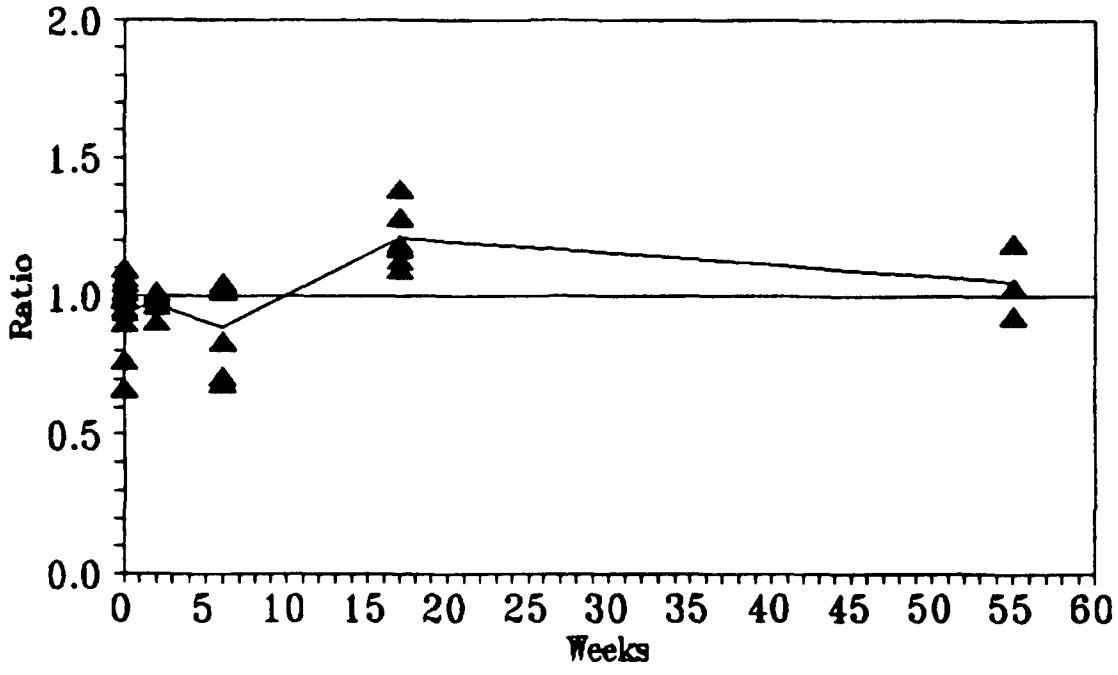

Figure 1. Ratio Plot of the Stability of the CTFE Trimer Carboxylic Acid Metabolite in Blood. The average value of the ratios is represented by the line plot.

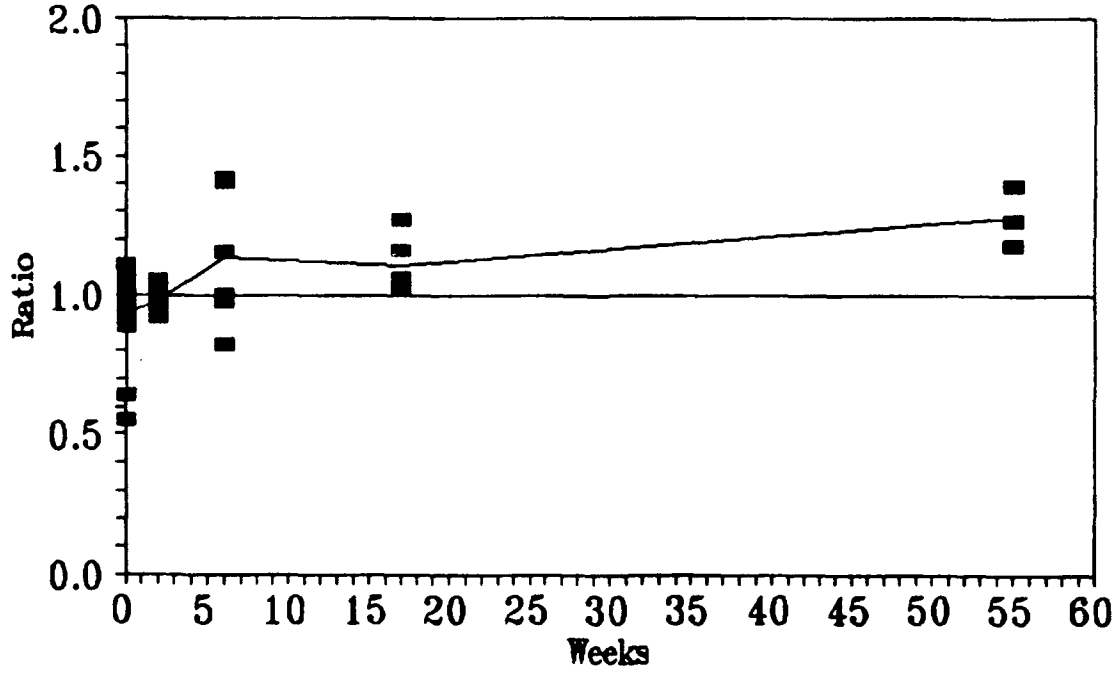

Figure 2. Ratio Plot of the Stability of the CTFE Tetramer Carboxylic Acid Metabolite in Blood. The average value of the ratios is represented by the line plot.

TABLE 3. pCTFE CARBOXYLIC ACIDS STABILITY: KIDNEY

Animal ID	Weeks Postsacrifice	Trimer Conc. in Kidney (µg/g)	Tetramer Conc. in Kidney (µg/g)	Trimer Day 0 Ratio	Tetramer Day 0 Ratio
Rat 001 Kidney	0	3.084	13.280	0.910	0.961
Rat 001 Kidney	0	4.009	15.451	1.183	1.118
Rat 001 Kidney	0	3.077	12.742	0.908	0.922
Rat 001 Kidney	2	3.661	14.634	1.080	1.059
Rat 001 Kidney	2	3.936	16.865	1.161	1.220
Rat 001 Kidney	2	3.690	14.936	1.089	1.081
Rat 001 Kidney	17	2.097	13.002	0.619	0.941
Rat 001 Kidney	17	2.373	14.823	0.700	1.072
Rat 001 Kidney	17	2.327	14.542	0.686	1.052
Rat 002 Kidney	0	2.855	9.706	1.053	1.052
Rat 002 Kidney	0	2.720	9.532	1.004	1.033
Rat 002 Kidney	0	2.556	8.446	0.943	0.915
Rat 002 Kidney	2	3.530	12.454	1.303	1.350
Rat 002 Kidney	2	3.539	11.909	1.306	1.291
Rat 002 Kidney	2	3.607	12.126	1.331	1.314
Rat 002 Kidney	17	1.884	9.687	0.695	1.050
Rat 002 Kidney	17	1.966	9.508	0.725	1.030
Rat 002 Kidney	17	1.885	8.416	0.696	0.912
Rat 003 Kidney	0	3.638	12.335	0.972	0.984
Rat 003 Kidney	0	3.537	11.041	0.945	0.881
Rat 003 Kidney	0	4.050	14.226	1.082	1.135
Rat 003 Kidney	2	4.078	14.403	1.090	1.149
Rat 003 Kidney	2	4.345	15.318	1.161	1.222
Rat 003 Kidney	2	4.611	16.563	1.232	1.322
Rat 003 Kidney	17	2.808	13.197	0.751	1.053
Rat 003 Kidney	17	2.770	12.909	0.740	1.030
Rat 003 Kidney	17	2.533	11.600	0.677	0.926
Rat 004 Kidney	0	3.901	9.756	1.074	1.074
Rat 004 Kidney	0	3.786	9.166	1.043	1.009
Rat 004 Kidney	0	3.208	8.321	0.883	0.916
Rat 004 Kidney	6	3.023	8.579	0.833	0.945
Rat 004 Kidney	6	3.197	9.464	0.880	1.042
Rat 004 Kidney	55	3.370	11.340	0.929	1.249
Rat 004 Kidney	55	3.110	10.410	0.856	1.146
Rat 004 Kidney	55	3.400	11.440	0.936	1.260
Rat 005 Kidney	0	1.819	8.216	1.048	1.063

(continued)

TABLE 3. Continued

Animal ID	Weeks Postsacrifice	Trimer Conc. in Kidney (µg/g)	Tetramer Conc. in Kidney (µg/g)	Trimer Day 0 Ratio	Tetramer Day 0 Ratio
Rat 005 Kidney	0	1.738	7.766	1.002	1.005
Rat 005 Kidney	0	1.649	7.198	0.950	0.932
Rat 005 Kidney	6	1.438	7.046	0.829	0.912
Rat 005 Kidney	6	1.717	8.932	0.990	1.156
Rat 005 Kidney	55	1.750	9.060	1.008	1.173
Rat 005 Kidney	55	1.600	8.170	0.922	1.057
Rat 005 Kidney	55	1.700	8.770	0.979	1.135
Rat 006 Kidney	0	3.136	14.779	1.028	1.040
Rat 006 Kidney	0	2.903	13.238	0.952	0.932
Rat 006 Kidney	0	3.110	14.606	1.020	1.028
Rat 006 Kidney	6	4.156	16.965	1.363	1.194
Rat 006 Kidney	6	3.432	16.748	1.125	1.179
Rat 006 Kidney	55	3.220	16.470	1.056	1.159
Rat 006 Kidney	55	3.310	16.900	1.085	1.189
Rat 006 Kidney	55	3.180	16.800	1.043	1.182

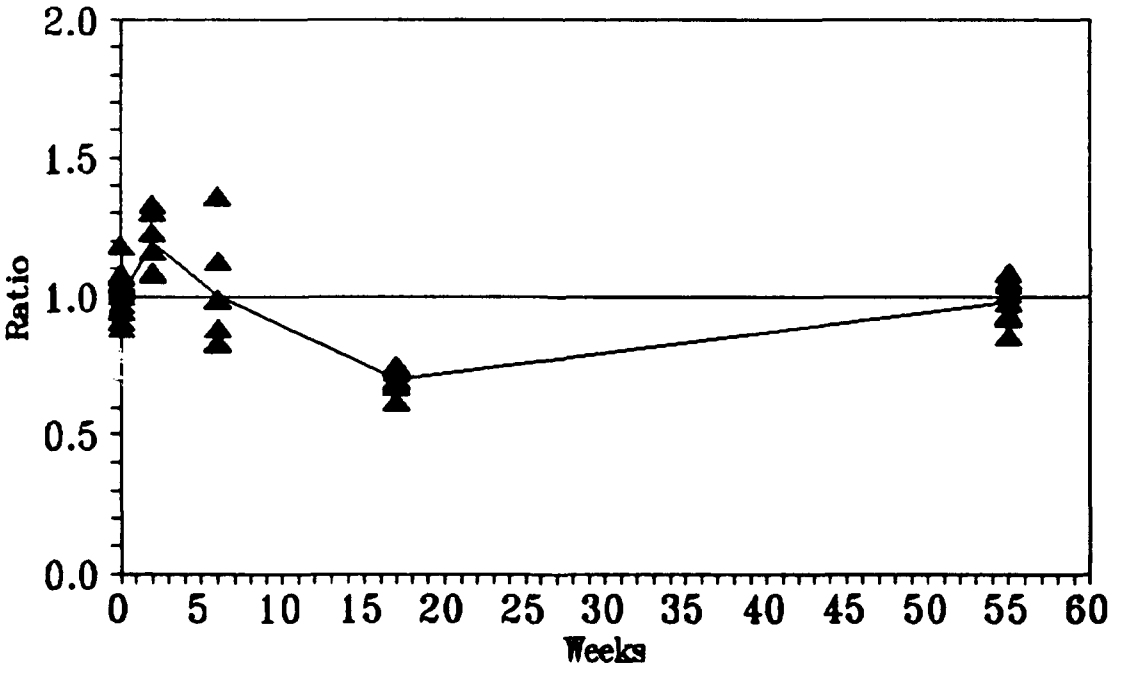

Figure 3. Ratio Plot of the Stability of the CTFE Trimer Carboxylic Acid Metabolite in Kidney. The average value of the ratios is represented by the line plot.

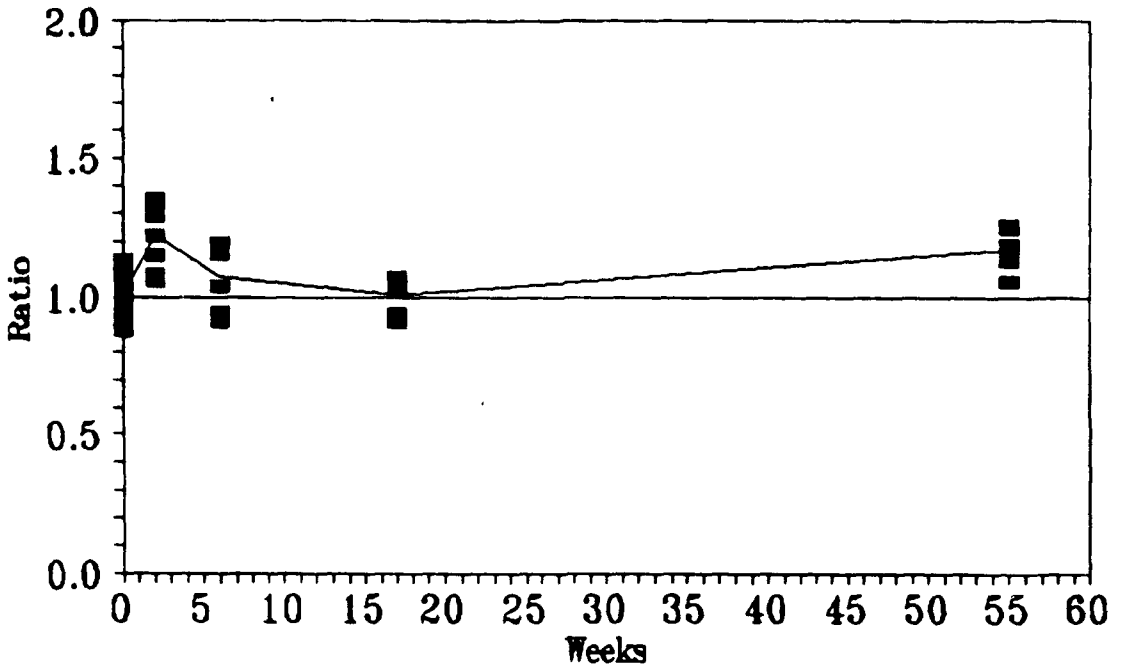

Figure 4. Ratio Plot of the Stability of the CTFE Tetramer Carboxylic Acid Metabolite in Kidney. The average value of the ratios is represented by the line plot.

The CTFE trimer carboxylic acid metabolite in kidney had an increase at 2 weeks, followed by a decrease from Weeks 6 to 17. At Week 55, there was an increase. The significance level of this trend was $p<0.05$. The CTFE tetramer carboxylic acid metabolite in kidney showed no significant trend.

The trimer and tetramer carboxylic acid metabolites of CTFE were found in liver. The results of these analyses are shown in Table 4. The stability plots for the CTFE trimer and tetramer carboxylic acid metabolites are shown in Figure 5 and Figure 6, respectively.

The CTFE trimer carboxylic acid metabolite in liver increased over the first 2 weeks, followed by a decrease from Weeks 6 to 17. This was followed by an increase at Week 55. The significance level of this trend was $p<0.01$. The CTFE tetramer carboxylic acid metabolite showed no significant trend.

The trimer and tetramer carboxylic acid metabolites of CTFE were found in lung. The results of these analyses are shown in Table 5. The stability plots for the CTFE trimer and tetramer carboxylic acid metabolites are shown in Figure 7 and Figure 8, respectively.

In lung tissue, the CTFE trimer carboxylic acid metabolite showed no significant trend. However, the CTFE tetramer carboxylic acid metabolite demonstrated a significant decrease ($p<0.01$).

TABLE 4. pCTFE CARBOXYLIC ACIDS STABILITY: LIVER

Animal ID	Weeks Postsacrifice	Trimer Conc. in Liver (µg/g)	Tetramer Conc. in Liver (µg/g)	Trimer Day 0 Ratio	Tetramer Day 0 Ratio
Rat 001 Liver	0	5.644	14.023	0.996	1.014
Rat 001 Liver	0	5.725	13.928	1.011	1.007
Rat 001 Liver	0	5.627	13.541	0.993	0.979
Rat 001 Liver	2	6.036	15.175	1.065	1.097
Rat 001 Liver	2	6.028	15.361	1.064	1.111
Rat 001 Liver	2	5.511	13.361	0.973	0.966
Rat 001 Liver	17	3.612	13.914	0.638	1.006
Rat 001 Liver	17	3.838	12.694	0.678	0.918
Rat 001 Liver	17	3.991	13.288	0.704	0.961
Rat 002 Liver	0	4.474	10.913	1.007	0.991
Rat 002 Liver	0	4.640	11.856	1.044	1.077
Rat 002 Liver	0	4.216	10.253	0.949	0.931
Rat 002 Liver	2	4.412	10.877	0.993	0.988
Rat 002 Liver	2	5.688	14.178	1.280	1.288
Rat 002 Liver	2	5.479	13.276	1.233	1.206
Rat 002 Liver	17	3.258	8.092	0.733	0.735
Rat 002 Liver	17	3.630	8.659	0.817	0.787
Rat 002 Liver	17	3.572	9.113	0.804	0.828
Rat 003 Liver	0	5.597	13.459	1.031	1.046
Rat 003 Liver	0	5.594	13.433	1.031	1.044
Rat 003 Liver	0	5.088	11.716	0.938	0.910
Rat 003 Liver	2	5.703	13.615	1.051	1.058
Rat 003 Liver	2	5.913	14.373	1.090	1.117
Rat 003 Liver	2	5.806	13.804	1.070	1.073
Rat 003 Liver	17	3.615	8.981	0.666	0.698
Rat 003 Liver	17	4.810	13.721	0.887	1.066
Rat 003 Liver	17	4.312	10.636	0.795	0.826
Rat 004 Liver	0	3.892	8.735	0.912	0.870
Rat 004 Liver	0	4.439	10.587	1.040	1.055
Rat 004 Liver	0	4.473	10.786	1.048	1.075
Rat 004 Liver	6	3.965	9.161	0.929	0.913
Rat 004 Liver	6	3.992	9.385	0.935	0.935
Rat 004 Liver	55	3.880	10.880	0.909	1.084
Rat 004 Liver	55	3.600	10.140	0.843	1.010
Rat 004 Liver	55	3.950	11.280	0.925	1.124
Rat 005 Liver	0	4.294	9.396	1.073	1.114

(continued)

TABLE 4. Continued

Animal ID	Weeks Postsacrifice	Trimer Conc. in Liver (µg/g)	Tetramer Conc. in Liver (µg/g)	Trimer Day 0 Ratio	Tetramer Day 0 Ratio
Rat 005 Liver	0	3.979	8.388	0.994	0.994
Rat 005 Liver	0	3.735	7.527	0.933	0.892
Rat 005 Liver	6	3.166	7.195	0.791	0.853
Rat 005 Liver	6	3.919	9.833	0.979	1.166
Rat 005 Liver	55	3.160	8.560	0.789	1.015
Rat 005 Liver	55	3.510	9.380	0.877	1.112
Rat 005 Liver	55	3.370	9.020	0.842	1.069
Rat 006 Liver	0	5.585	13.550	1.011	1.005
Rat 006 Liver	0	5.700	14.063	1.032	1.043
Rat 006 Liver	0	5.290	12.845	0.957	0.952
Rat 006 Liver	6	6.765	14.659	1.225	1.087
Rat 006 Liver	6	5.359	10.460	0.970	0.776
Rat 006 Liver	55	5.180	15.400	0.938	1.142
Rat 006 Liver	55	5.180	15.910	0.938	1.180
Rat 006 Liver	55	5.310	16.020	0.961	1.188

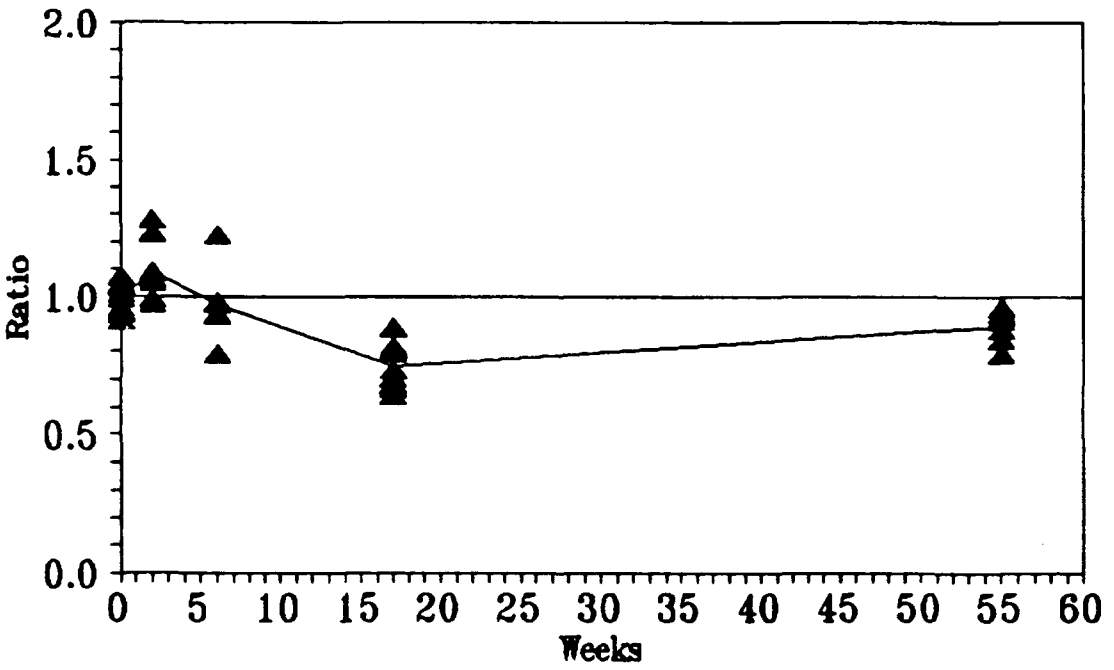

Figure 5. Ratio Plot of the Stability of the CTFE Trimer Carboxylic Acid Metabolite in Liver. The average value of the ratios is represented by the line plot.

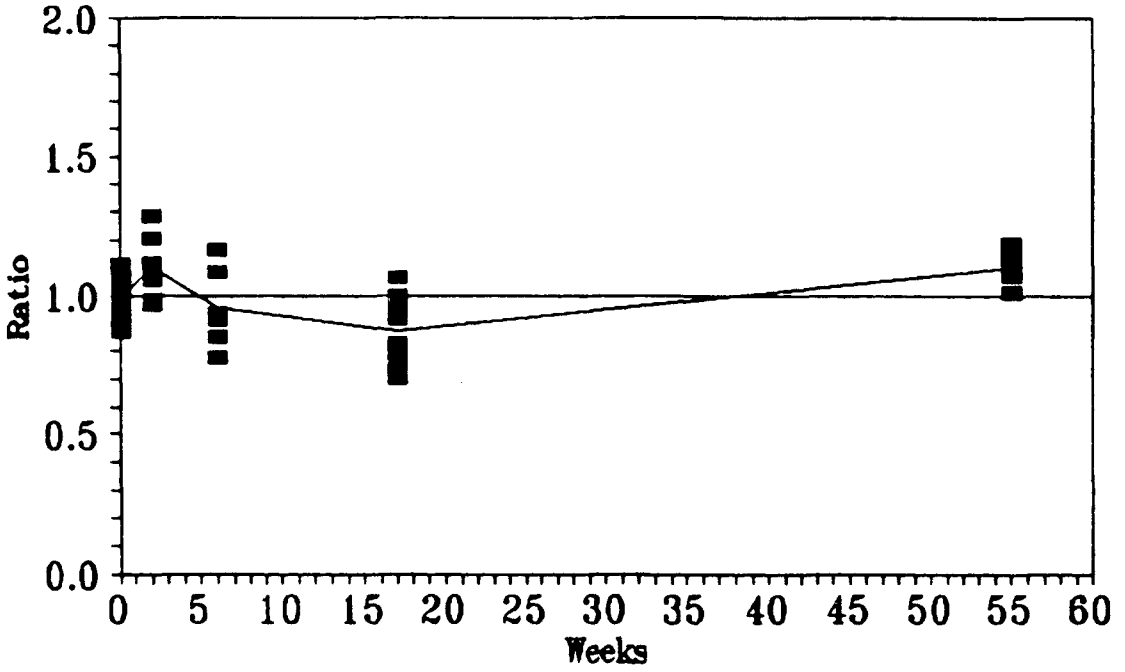

Figure 6. **Ratio Plot of the Stability of the CTFE Tetramer Carboxylic Acid Metabolite in Liver.** The average value of the ratios is represented by the line plot.

TABLE 5. pCTFE CARBOXYLIC ACIDS STABILITY: LUNG

Animal ID	Weeks Postsacrifice	Trimer Conc. in Lung (µg/g)	Tetramer Conc. in Lung (µg/g)	Trimer Day 0 Ratio	Tetramer Day 0 Ratio
Rat 001 Lung	0	2.482	5.042	1.006	0.989
Rat 001 Lung	0	2.450	5.155	0.994	1.011
Rat 001 Lung	2	2.432	7.459	0.986	1.463
Rat 001 Lung	2	2.533	7.850	1.027	1.540
Rat 001 Lung	17	2.025	6.801	0.821	1.334
Rat 001 Lung	17	2.417	6.722	0.980	1.318
Rat 002 Lung	0	2.537	5.061	0.993	1.012
Rat 002 Lung	0	2.571	4.940	1.007	0.988
Rat 002 Lung	2	3.075	6.238	1.204	1.248
Rat 002 Lung	2	3.437	7.131	1.346	1.426
Rat 002 Lung	17	2.209	4.279	0.865	0.856
Rat 002 Lung	17	2.587	5.174	1.013	1.035
Rat 003 Lung	0	2.404	4.357	0.969	0.955
Rat 003 Lung	0	2.559	4.765	1.031	1.045
Rat 003 Lung	2	3.038	5.990	1.224	1.313
Rat 003 Lung	2	3.030	5.979	1.221	1.311

(continued)

TABLE 5. Continued

Animal ID	Weeks Postsacrifice	Trimer Conc. in Lung (µg/g)	Tetramer Conc. in Lung (µg/g)	Trimer Day 0 Ratio	Tetramer Day 0 Ratio
Rat 003 Lung	17	1.840	4.848	0.741	1.063
Rat 003 Lung	17	2.100	5.411	0.846	1.186
Rat 004 Lung	0	1.648	3.119	1.020	1.017
Rat 004 Lung	0	1.583	3.016	0.980	0.983
Rat 004 Lung	6	1.615	2.826	1.000	0.921
Rat 004 Lung	6	1.504	2.509	0.931	0.818
Rat 005 Lung	0	1.565	3.654	0.991	1.005
Rat 005 Lung	0	1.593	3.616	1.009	0.995
Rat 005 Lung	6	1.410	2.887	0.892	0.794
Rat 005 Lung	6	1.558	3.377	0.987	0.929
Rat 006 Lung	0	2.744	6.926	1.050	1.068
Rat 006 Lung	0	2.480	6.043	0.950	0.932
Rat 006 Lung	6	1.870	4.553	0.716	0.702
Rat 006 Lung	6	2.015	4.553	0.772	0.702
Rat 006 Lung	55	2.230	5.720	0.854	0.882
Rat 006 Lung	55	2.050	5.130	0.785	0.791

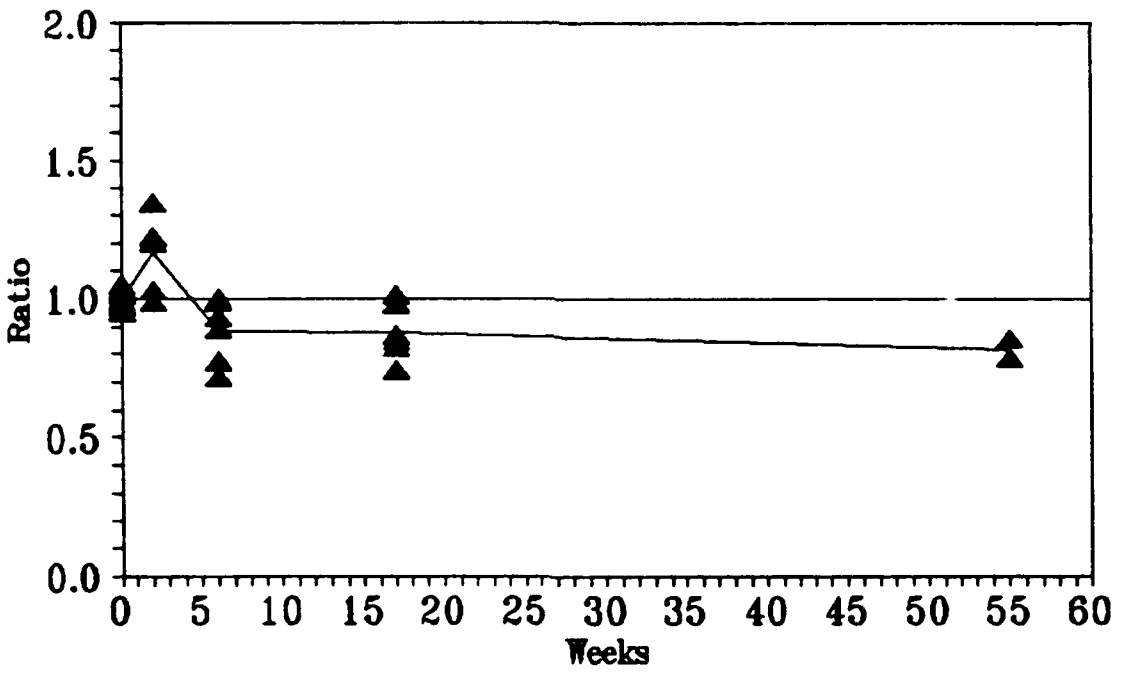

Figure 7. Ratio Plot of the Stability of the CTFE Trimer Carboxylic Acid Metabolite in Lung. The average value of the ratios is represented by the line plot.

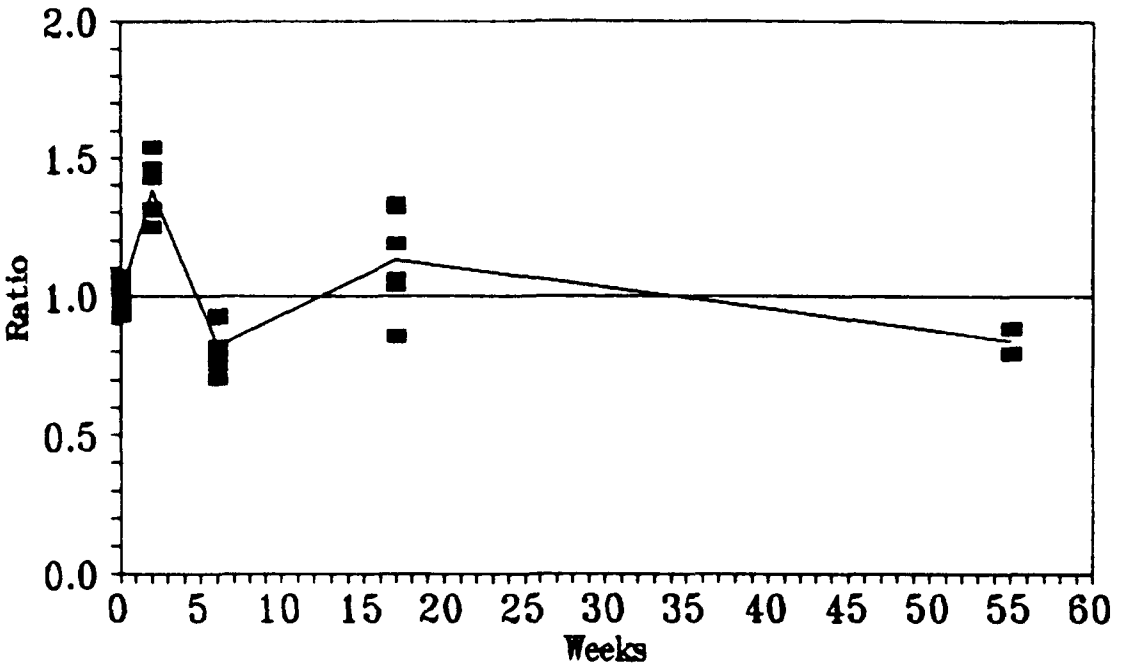

Figure 8. Ratio Plot of the Stability of the CTFE Tetramer Carboxylic Acid Metabolite in Lung. The average value of the ratios is represented by the line plot.

The trimer and tetramer carboxylic acid metabolites of CTFE were found in urine. The results of these analyses are shown in Table 6. The stability plots for the CTFE trimer and tetramer carboxylic acid metabolites are shown in Figure 9 and Figure 10, respectively.

In urine, the CTFE trimer carboxylic acid metabolite showed no significant trend. The CTFE tetramer carboxylic acid metabolite showed a slight decrease between Weeks 2 and 6. This was followed by an increase at 17 weeks, which leveled off at 55 weeks. The significance level of this trend was $p<0.01$.

TABLE 6. pCTFE CARBOXYLIC ACIDS STABILITY: URINE

Animal ID	Weeks Postsacrifice	Trimer Conc. in Urine (µg/mL)	Tetramer Conc. in Urine (µg/mL)	Trimer Day 0 Ratio	Tetramer Day 0 Ratio
Rat 001 Urine	0	29.559	0.530	1.047	1.085
Rat 001 Urine	0	26.903	0.447	0.953	0.915
Rat 001 Urine	2	27.158	0.547	0.962	1.119
Rat 001 Urine	2	23.451	0.417	0.831	0.853
Rat 001 Urine	17	18.741	0.629	0.664	1.288
Rat 001 Urine	17	19.795	0.629	0.701	1.287
Rat 002 Urine	0	20.106	0.295	1.004	1.030
Rat 002 Urine	0	19.942	0.277	0.996	0.970
Rat 002 Urine	2	16.063	0.242	0.802	0.846
Rat 002 Urine	2	14.740	0.219	0.736	0.767
Rat 002 Urine	17	14.097	0.328	0.704	1.145
Rat 002 Urine	17	14.510	0.326	0.725	1.138
Rat 003 Urine	0	24.589	0.341	1.048	1.112
Rat 003 Urine	0	22.325	0.272	0.952	0.888
Rat 003 Urine	2	25.971	0.448	1.107	1.462
Rat 003 Urine	2	25.790	0.426	1.099	1.390
Rat 003 Urine	17	21.886	0.732	0.933	2.388
Rat 003 Urine	17	19.452	0.639	0.829	2.084
Rat 004 Urine	0	15.418	0.239	0.963	0.991
Rat 004 Urine	0	16.590	0.243	1.037	1.009
Rat 004 Urine	6	16.893	0.276	1.056	1.148
Rat 004 Urine	6	15.283	0.225	0.955	0.936
Rat 004 Urine	55	15.800	0.348	0.987	1.445
Rat 004 Urine	55	16.900	0.331	1.056	1.375
Rat 004 Urine	55	17.000	0.411	1.062	1.707
Rat 005 Urine	0	15.309	0.310	1.058	1.034
Rat 005 Urine	0	13.627	0.289	0.942	0.966
Rat 005 Urine	6	13.803	0.302	0.954	1.008
Rat 005 Urine	6	10.142	0.266	0.701	0.888
Rat 005 Urine	55	19.200	0.599	1.327	1.999
Rat 005 Urine	55	20.500	0.560	1.417	1.869
Rat 005 Urine	55	20.900	0.564	1.445	1.882
Rat 006 Urine	0	24.421	0.471	1.017	1.015
Rat 006 Urine	0	23.599	0.457	0.983	0.985
Rat 006 Urine	6	25.199	0.440	1.050	0.948
Rat 006 Urine	6	21.450	0.376	0.893	0.811
Rat 006 Urine	55	25.500	0.531	1.062	1.145
Rat 006 Urine	55	24.800	0.519	1.033	1.119
Rat 006 Urine	55	23.700	0.553	0.987	1.192

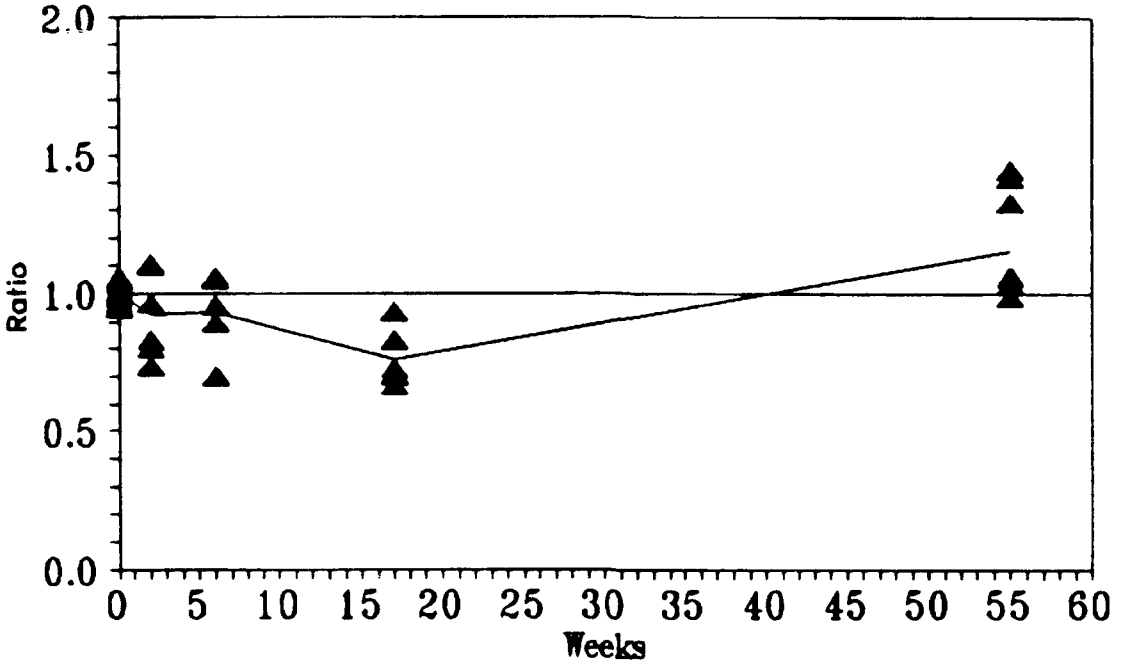

Figure 9. Ratio Plot of the Stability of the CTFE Trimer Carboxylic Acid Metabolite in Urine. The average value of the ratios is represented by the line plot.

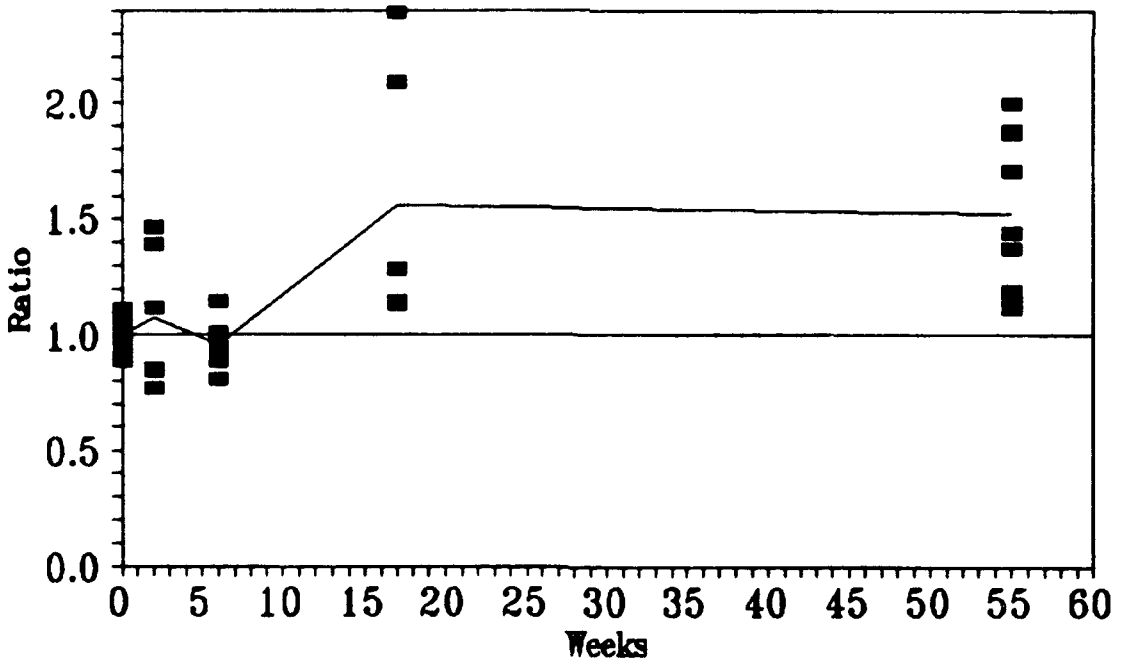

Figure 10. Ratio Plot of the Stability of the CTFE Tetramer Carboxylic Acid Metabolite in Urine. The average value of the ratios is represented by the line plot.

SECTION 4

DISCUSSION

Male F-344 rats were dosed orally (1.25 g/kg) with pCTFE parent compound. Urine and feces were collected from 24- to 48-h post dose. At 48-h post dose, the animals were sacrificed, and tissue samples were obtained. At the time of sacrifice, the urine, feces, and tissue samples were immediately analyzed for carboxylic acid metabolites of pCTFE. To evaluate storage stability, the remainder of each sample was frozen for serial analysis.

Samples were analyzed at 2, 6, 17, and 55 weeks. In blood, the CTFE trimer carboxylic acid metabolite did not change significantly. However, the tetramer carboxylic acid metabolite increased, decreased, and then increased slowly. The CTFE trimer carboxylic acid metabolite in kidney and in liver exhibited an increase, followed by a decrease, and then increased at 55 weeks. The tetramer carboxylic acid metabolite did not change significantly in kidney or liver. In lung, the CTFE trimer carboxylic acid metabolite did not change significantly. However, the CTFE tetramer carboxylic acid metabolite decreased over the time-course of the study. In urine, the trimer carboxylic acid metabolite did not change significantly, but the tetramer carboxylic acid metabolite decreased, and then increased, before leveling off at 17 weeks and 55 weeks.

These trends are predominantly a combination of increases and decreases in the concentrations of the pCTFE carboxylic acid metabolites. Degradation of the metabolites would produce a decreasing trend. Concentration of the metabolites from the evaporation of water would produce an increasing trend. During the duration of the experiments, the urinary levels of the CTFE tetramer carboxylic acid metabolite appeared to increase. However, this same trend was not reflected in the more concentrated CTFE trimer carboxylic acid metabolite. A statistically significant decrease was found for CTFE tetramer in lung. However, a corresponding decrease was not found in the concentrations of CTFE trimer carboxylic acid metabolite in lung. Although degradation and concentration effects may be present, the predominant effect is most probably analytical variation. Some sources of analytical variation are error in measuring the amount of sample for analysis, variation in the reaction efficiency for the derivatization, and variation in the extraction efficiency of the carboxylic acid metabolites.

The pCTFE carboxylic acid metabolite study demonstrates that the trimer and tetramer carboxylic acid metabolites can be measured in tissue samples after being stored for a period of at least 1 year. The study also demonstrates that analytical variation can be significant, and that the direct comparison of analytical data generated weeks apart should be done with caution. One step that could reduce this day-to-day analytical variation would be to store tissue samples from a control animal, which contain a known amount of added pCTFE carboxylic acids, with tissue samples to be

analyzed. The analysis of this control sample would provide an estimate of the analytical variation. This could be used to generate a correction factor for the data.

In conclusion, the CTFE trimer and tetramer carboxylic acid metabolites in urine can be detected after being stored for 55 weeks at -20 °C, and in tissues and blood at -70 °C. None of the samples analyzed had pCTFE carboxylic acid metabolite concentrations that decreased to nondetectable levels. The lack of a consistent decrease in the tissue concentrations indicates that degradation of the metabolites is not significant. The lack of a consistent increase in the concentration of the metabolites rules out a significant lyophilization effect. The day-to-day variation of the analytical methodology appears to be responsible for the observed variability in the biological samples assayed.

SECTION 5

REFERENCES

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QUALITY ASSURANCE

The study, "Storage Stability of pCTFE Carboxylic Acid Metabolites," was conducted by the ManTech Environmental Technology, Inc., Toxic Hazards Research Unit under the guidance of the Environmental Protection Agency's Good Laboratory Practices Standards, 40 CFR 792. No claim will be made that this was a "GLP" study as no attempt was made to adhere to the strict requirements of those standards.

The various phases of this study were inspected by members of the Quality Assurance Unit. Results of the inspections were reported directly to the Study Director at the close of each inspection.

DATE OF INSPECTION	ITEM INSPECTED
February 11, 1991	Dosing of animal Group I
February 13, 1991	Sacrifice and tissue harvest from animal Group I
February 27, 1991	Specimen assay after 2 weeks storage
December 23, 1991	Specimen assay after 10 months storage
August 7 - September 2, 1992	Data and final report audit
September 14, 1992	Review of audit results and corrective actions

The Quality Assurance Unit has determined through review process that this report accurately describes those methods and standard operating procedures required by the protocol and that the reported results accurately reflect the raw data obtained during the course of the study. No discrepancies were found that would alter the interpretations presented in this Final Report.

M. G. Schneider
QA Coordinator
Toxic Hazards Research Unit

Date 14 September 1992